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Chemoreceptors of Crustaceans: Similarities to Receptors for Neuroactive Substances in Internal Tissues

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A description is given of crustacean chemosensory systems and the neurophysiological procedures used to study them. Their response properties and tuning characteristics are discussed. A review is then provided of specific crustacean chemoreceptors that are stimulated selectively by either purine nucleotides, taurine, glutamate, or glycine, all of which have neuroactive properties in internal tissues.

Two distinctly different types of purinergic chemoreceptors occur on the antennules of the spiny lobster. P_1-like chemoreceptors have a potency sequence of AMP > ADP > ATP > adenosine and show a strict structural requirement for the ribose phosphate moiety. P_2-like chemoreceptors have a potency sequence of ATP > ADP > AMP or adenosine and show a broad sensitivity to nucleotide triphosphates with modifications in both the purine and ribose phosphate moieties. Sensilla containing the dendrites of chemosensory neurons also possess an ectonucleotidase(s) that inactivates excitatory nucleotides to yield adenosine which is subsequently internalized by a sensillar uptake system.

Narrowly tuned taurinergic chemoreceptors are present on both the antennules and legs of lobsters. Although taurine itself is the most effective stimulant, the taurine analogs hypotaurine and β-alanine are also very excitatory. Structure-activity studies indicate these chemoreceptors have marked similarities to taurine-sensitive systems in internal tissues of vertebrates. By contrast, comparative studies of glutamatergic chemoreceptors on the legs of lobsters indicate response spectra different from those of the glutamate receptors in lobster neuromuscular junctions and the three classes of excitatory amino acid receptors identified internally in vertebrates. Crustacean chemoreceptors for glycine, ecdysteroids, and pyridine are also described. The hypothesis that receptors for internal neuroactive agents may have originally evolved as external chemoreceptors of primitive aquatic organisms is discussed.

Introduction

The chemical senses of many aquatic animals are extremely well developed. Waterborne chemicals influence facets of behavior as diverse as food finding (*1,2*), predator avoidance (*3,4*), mate recognition and mating (*5,6*), substrate selection and metamorphosis by larvae (*7,8*), homing by migratory species (*9,10*), and species recognition and social behavior (*11,12*). Chemoreception in aquatic animals has been reviewed recently (*3,13–15*).

Chemosensory studies have revealed that many of the external chemical agents stimulating behavioral and physiological responses in aquatic invertebrates are the very same substances that function internally in higher organisms as either transmitters or modulators. This paper will briefly describe crustacean chemosensory systems and the physiological procedures used to study them. A description will then be provided of specific crustacean chemoreceptors that are stimulated by substances known to have neuroactive properties in internal tissues.

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Chemosensory Systems in Decapod Crustaceans

Because of their large size and easily accessible chemosensory organs, certain decapod crustaceans such as the Florida spiny lobster (*Panulirus argus*), the California spiny lobster (*P. interruptus*), the American lobster (*Homarus americanus*), and certain crayfish species (e.g., *Orconectes limosus*) have proven to be popular animals for neurophysiological studies of the chemosensory process. The functional organization of the olfactory system in these crustaceans has been reviewed recently (*1,16*). The olfactory organ consists of a tuft of sensilla, termed aesthetasc sensilla, present on the lateral filament of the antennule (Fig. 1). In the Florida spiny lobster, *P. argus*, each antennule has about 2000 of these sensilla, with each sensillum being approximately 1000 μm in length and 40 μm in diameter. Each is innervated by the cilia-bearing dendrites of an

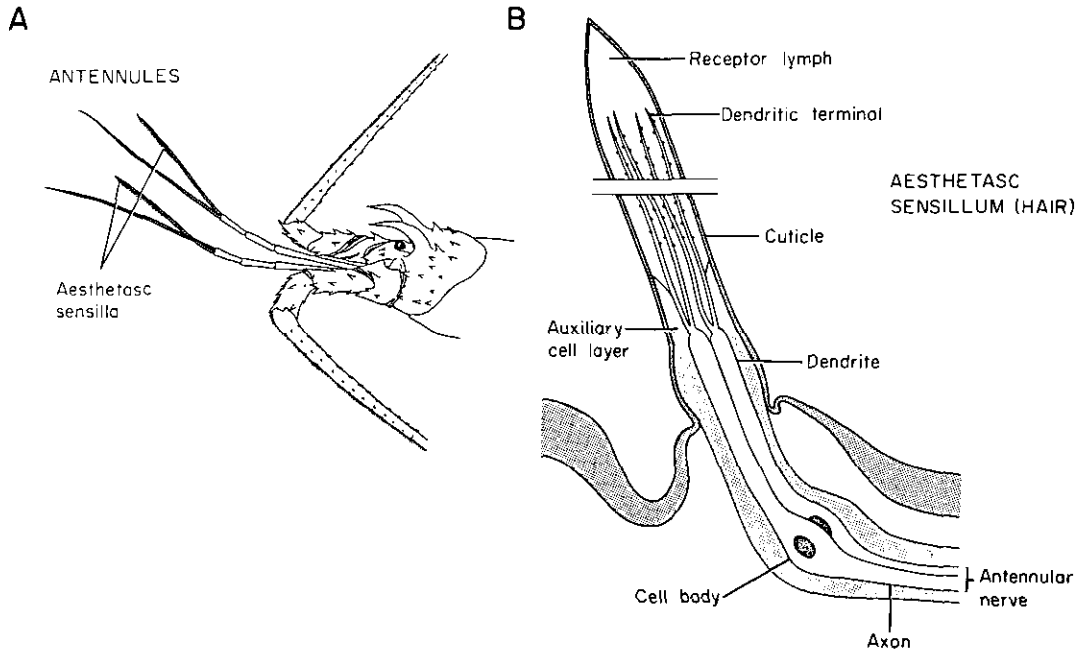

FIGURE 1. (*A*) Head of the spiny lobster showing the lateral branches of the antennules with tufts of chemosensory aesthetasc sensilla. (*B*) Schematic of aesthetasc sensillum depicting dendritic terminals housed within the cuticular sheath.

estimated 350 bipolar sensory neurons (*17*). Axons from these olfactory cells project via the antennular nerve to the midbrain where they make synaptic contacts in the glomerular neuropile of the paired olfactory lobes (*18,19*).

In addition to the aesthetasc sensilla of the antennules, a variety of other morphological types of chemosensory sensilla have been identified on the mouth parts, claws and walking legs of decapod crustaceans (*20–23*). Functionally these other chemoreceptors are often considered to be comparable to the taste system of vertebrates (*1,24*). The chemical sensitivities of receptors on the distal segments (dactyls) of the walking legs have been especially well studied (*25–29*).

Procedures for Neurophysiological Analysis of Crustacean Chemoreceptors

The crustacean chemoreceptor cell is a primary sensory neuron with a spiking axon that projects directly to the brain. The responses of these cells to chemicals can be quantified by extracellularly recording the neural discharge of the axon when chemicals are applied to the dendrite. Such recordings are accomplished by mounting an excised chemosensory appendage in a chamber that allows the dendrite-bearing sensilla to be superfused with chemicals. One such arrangement for stimulating and recording from crustacean chemoreceptor cells is illustrated in Figure 2. Continuous perfusion with oxygenated saline maintains the viability of the preparation.

Chemical stimulation of the preparation in Figure 2 is achieved by injecting small volumes (i.e., 20 to 300 μL) of solutions, as a bolus, into a carrier flow of water that continuously flushes over the sensilla. This procedure introduces the stimulus with no disruption of flow concurrent with the arrival of the stimulus at the preparation. Depending on the volume of stimulant injected, the preparation can be exposed to either a brief (e.g., 3 sec) pulse, or to a prolonged plateau of a constant concentration of the stimulant. Conductivity and fluorometric analyses show that this procedure delivers reproducible profiles of stimulant that approximate a step function on their leading edge.

Extracellular recording from receptor cells is achieved by splitting fascicles from the nerve and applying suction electrodes en passant to the exposed axons. Chemosensitive units are identified as such by their altered frequency of discharge to brief pulses of search stimuli and their lack of response to identically applied control carrier water. Responses from single neurons are obtained either by fortuitous separation of fascicles containing only a single active unit or by window (voltage and/or time) discrimination of single units from multi-unit recordings. Single-cell status of all records can be verified by an on-line template matching scheme that involves passing the real time analog signal through a delay register to retain the pretriggered aspect of the waveform and superimposing the entire waveform of each spike that triggers the window discriminator on the synchronized display of an analog storage oscilloscope. Evoked responses (spike trains) can be quantified by electrically counting the output of the window discriminator if the parameter of interest is the total num-

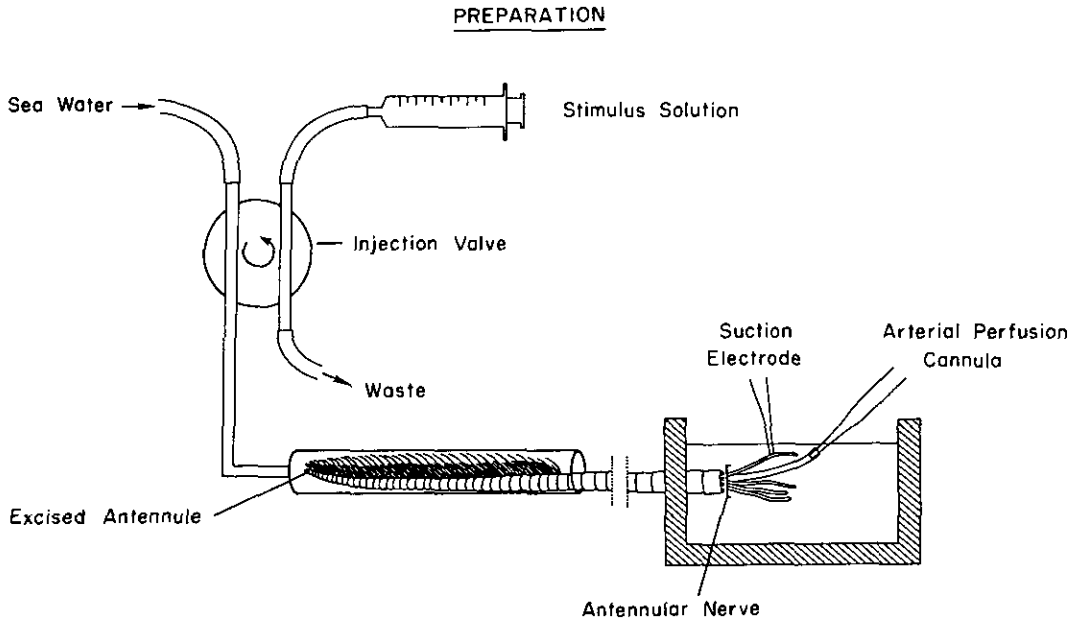

FIGURE 2. Diagram of a typical stimulus delivery and recording apparatus used to analyze crustacean chemoreceptor cells. An excised, perfused antennular preparation is illustrated; similar systems have been used to study dactyl chemoreceptors. Additional details are given in the text.

ber of spikes. Alternatively, a microprocessor-based interval analysis can be performed on the same output if it is necessary to obtain other, time-dependent parameters of the spike train.

Now it is also possible to record intracellularly from crustacean chemoreceptor cells (*30*). This technique is only beginning to be applied to the particular types of receptor cells discussed in this review, but the technology is now available to examine the biophysical basis of stimulus-response coupling in these cells.

Response Properties of Crustacean Chemoreceptors

Most crustacean chemoreceptor cells have very low levels of spontaneous activity ($\leq$ 1 Hz). Chemical stimuli cause these cells to show a tonic increase in the frequency of discharge, although the response often becomes slightly more phasic at higher stimulus concentrations (Fig. 3). In nature, most chemoreceptors are only exposed intermittently to a chemical stimulus, and the actual pattern of the response is strongly influenced by the dynamics of the access of the stimulus to the receptor surface. The lobster antennule, for example, is flicked reflexively at a rate of 1–2 Hz. This behavior appears to regulate the access of the stimulus to the tight cluster of sensilla; the stimulus is sampled as a series of discontinuous pulses that onset within about 100 msec. The afferent discharge of the sensory cells reflects this pulsatile stimulus profile as a series of brief, phasic bursts (*31*).

In a chemosensory cell, different stimulatory compounds usually evoke responses that have similar temporal patterns, although instances where the discharge pattern may change have been reported (*32*). Perhaps constancy in the temporal pattern of discharge with different stimulants is to be expected. The use of different temporal patterns to encode different stimulants would make it difficult to distinguish the pattern for a particular stimulant from patterning introduced by short-term fluctuations in stimulus concentration. The pulsatile nature of odorants in the aquatic environment has been described (*14*).

The rate of discharge of chemoreceptor cells is typically a simple function of the stimulus concentration (Fig. 3). The response of single cells usually saturates over 2 to 3 log units of concentration, although populations of cells reach saturation over broader ranges of concentration. The latter is presumably indicative of range fractionation which enables the overall population to be sensitive to a wide range of concentrations while still retaining maximal sensitivity to changes in concentration.

A striking feature of some crustacean chemoreceptors is their extremely low threshold. Antennular chemoreceptors in the spiny lobster give discrete responses to picomolar concentrations of some amino acids (*33*). Dactyl receptors in the American lobster respond to as little as 35 femtomolar ammonia (*25*).

"Tuning" Characteristics of Crustacean Chemoreceptors

"Tuning" refers to the number and the heterogeneity of the different compounds that are stimulatory to a

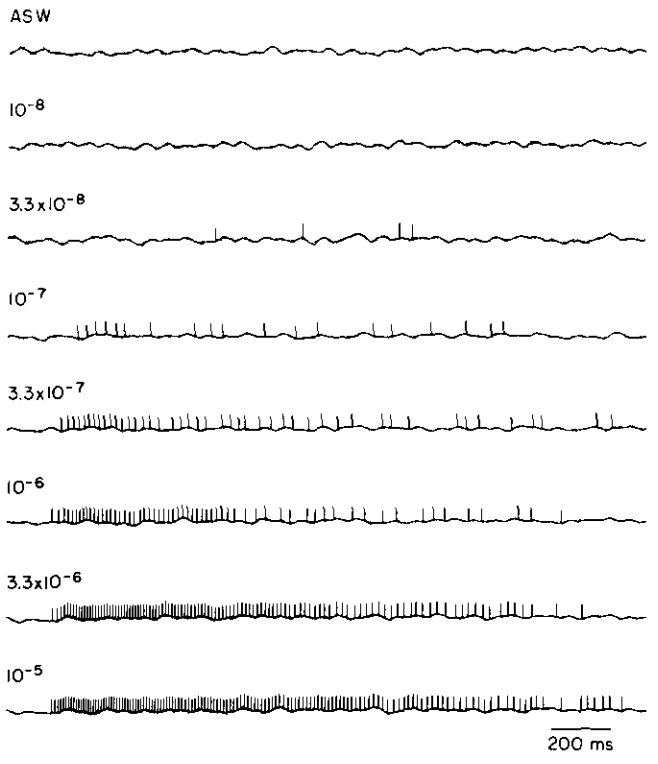

FIGURE 3. Effect of stimulus concentration on the neural discharge of a chemoreceptor cell from the antennule of the spiny lobster, *P. argus*. Shown are the extracellularly recorded spike trains evoked by a 2-sec exposure to taurine at the molar concentrations indicated, and to seawater (ASW). The traces faithfully reproduce the temporal pattern of the train, but not the waveform of the spike, which has been shaped electrically for maximum clarity. Note that concentration has little effect on the temporal pattern of the response, primarily altering the frequency and number of spikes in the train.

chemoreceptor cell; tuning characteristics of crustacean chemoreceptors have been reviewed recently (*34*). In crustaceans it is known that different types of cells contribute to the peripheral chemosensory organization. Chemoreceptor cells with narrow response spectra are found on the dactyls (*26,35*) and antennules (*36–38*) of lobsters. These cells are narrowly tuned to particular amino acids and nucleotides found in food organisms. Specific examples of these types of cells will be described later. Such narrowly tuned cells are often called "specialists," following terminology originally used by Schneider et al. (*39*) for moths. Other cells are less narrowly tuned, but are still limited in the breadth of their response. For example, the amino acid-selective units in the dactyls of the crayfish are limited in their response to a particular structural class of molecules, but do not appear to be selective within the class (*40*). Still other receptor cells are more broadly tuned and are sometimes called "generalists," again by the terminology proposed by Schneider et al. (*39*). Broadly tuned receptor neurons respond to an array of structurally different substances that belong to different functional classes of molecules. In some broadly tuned neurons, sensitivity to different molecules is not necessarily distributed randomly across the receptor population. When relatively large numbers of potentially stimulatory compounds have been tested on individual receptor cells in insects, for example, the cells tend to fall into "reaction groups." Each group is composed of cells having part, or all, of their response spectra more similar to that of other cells in the same reaction group than to cells in other groups (*41*).

Purinergic Chemoreceptors

Background

Cyclic adenosine 3′,5′-monophosphate has long been known to function both as a potent chemoattractant in cellular slime molds (*42*) and as a second messenger in many physiological processes (*43,44*). Regarding vertebrate organisms, adenosine and its noncyclic nucleotides are known to play major roles as transmitters or modulators of physiological activities in the nervous system, the vascular system, and in the smooth muscle of many internal organs (*45–51*). The purinergic receptors that mediate responses to adenosine (Ado) and adenine nucleotides have been classified by Burnstock (*52*) as P_1 and P_2 types. Among the distinguishing features of these two receptor types are the following: P_1-type purinoceptors have a potency sequence of Ado $\geq$ AMP $>$ ADP $\geq$ ATP, whereas the P_2-type has a potency sequence of ATP $\geq$ ADP $>$ AMP $\geq$ Ado. It will be shown below that marine crustaceans possess olfactory chemosensory cells with marked similarities to both the P_1- and the P_2-type purinoceptors found in internal tissues.

P_1-like Purinergic Chemoreceptors

The first evidence that P_1-like chemoreceptors are present in crustaceans came from the behavioral observation that adenosine 5′-monophosphate (AMP) was a potent attractant for the grass shrimp, *Palaemonetes pugio*. Bioassays of AMP analogs suggested that the behavioral response of the shrimp is mediated by receptors akin to P_1-type purinoceptors (*53*).

Documentation of the existence of purinergic chemoreceptors in crustaceans was obtained in physiological studies with the spiny lobster, *P. argus*, by employing the procedures for single-cell, extracellular recording as described above for ablated antennular filaments. Chemosensory purinoceptors with a potency sequence of AMP $>$ ADP $>$ ATP $>$ Ado are present in the lobster antennule (Fig. 4) (*37*); this potency sequence, measured physiologically, is the same as that measured behaviorally in the shrimp. Studies of structure-activity relationships (SAR) of AMP and 16 analogs (Fig. 5) show that AMP is the most potent stimulant, and all changes in its structure result in a significant diminution in activity. However the greatest diminution accompanies changes in the ribose phosphate moiety rather than the adenine moiety. This is seen in Figure 5 by noting that eight of the nine least active analogs are those with modifications in the ribose phosphate

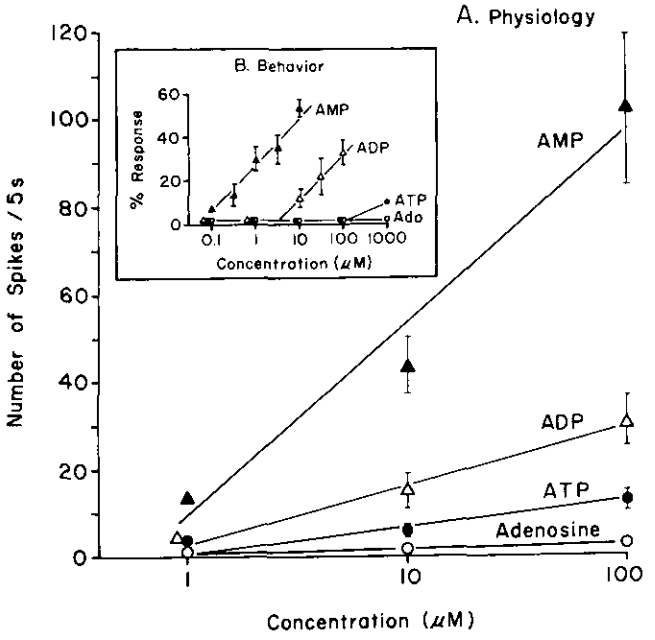

FIGURE 4. (*A*) Dose-response relationships for adenine nucleotides and adenosine as measured physiologically in chemosensory neurons of the spiny lobster. (*B*) (Inset) Behavioral results obtained with same substances in a shrimp. In both cases the potency sequence was AMP > ADP > ATP > adenosine. Data points are *mean values* (± SEM). *From Derby et al. (37).*

moiety. 8-bromo-AMP, the least active adenine-modified analog, differs from the others by having a *syn*- rather than an *anti*-conformation (*54*). Both the physiological response of the lobster chemoreceptors and the behavioral response of the shrimp are antagonized by the methylxanthine, theophylline.

Vertebrate P_1-type (or R-type) purinoceptors generally have the following characteristics: (1) show a potency sequence of Ado ≥ AMP > ADP ≥ ATP; (2) are most affected by structural changes in the ribose moiety of the stimulant; (3) prefer agonists in the *anti*-conformation; and (4) are antagonized by theophylline and other methylxanthines (*52,55,56*). Hence the only major difference between the P_1-type purinoceptor and P_1-like chemoreceptors of the lobster (and the grass shrimp) is that the chemoreceptor requires the presence of the 5′-phosphate group on the nucleoside. The 5′-phosphate group is apparently optional for activation of the P_1-type receptor of vertebrates.

In addition to the crustaceans described above, there are other animals possessing external chemoreceptors for certain nucleotides or nucleosides. The African armyworm, an insect larva, has a receptor for adenosine that may function in feeding behavior (*57*). Also, AMP is an attractant for larval mosquitos (*58*). Further, receptors on the lips of a fish, the puffer, respond preferentially to nucleotides, especially ADP, IMP, and UMP (*59*). Likewise, IMP and inosine are gustatory feeding stimulants in another fish, the turbot (*60*). Nucleotides have a different type of effect on external chemoreceptors found in bovine taste papillae. In this case, the nucleotides GMP, IMP, and UMP increase the amount of glutamate bound to glutamate receptors (*61*).

P_2-like Purinergic Chemoreceptors

In addition to the P_1-like chemoreceptors described above, the spiny lobster has a second distinct population of purinergic chemoreceptors with response characteristics indicative of the P_2-type purinoceptors found in internal tissues of vertebrates (*38*). This second population is composed of ATP-sensitive cells with response properties easily distinguished from the AMP-sensitive (P_1-like) cells described earlier (Fig. 6). Responses given by the ATP-sensitive cells are very brief in duration (less than 0.5 sec), even at high concentrations, whereas the AMP-sensitive cells typically have response durations of 5 to 10 sec, or longer (Fig. 6). Also, the maximum discharges of the ATP-sensitive cells to ATP are of low magnitude (ca. 11 impulses/response, SEM ± 0.95) whereas the AMP-sensitive cells have high maximum discharges (ca. 104 impulses/response, SEM ± 24.5).

SAR studies of the ATP-sensitive cells were conducted with the nucleotides and other substances shown in Figure 7. In these cells, ATP is far more active than ADP, whereas AMP and Ado are virtually nonstimulatory. An activity sequence for other substances is as follows: ATP ≥ deoxyATP (dATP) > GTP > CTP ≥ XTP ≥ ITP >> 8-bromo-ATP = ADP, with pyrophosphate and tripolyphosphate being virtually inactive. Hence, these cells possess a broad sensitivity to nucleotide triphosphates and tolerate changes in both the ribose and adenine moieties. The minor differences in activity seen with most nucleotide triphosphates contrasts dramatically with a ca. 20-fold decrease in activity seen when the diphosphate group of ADP is substituted for the triphosphate chain of ATP.

The ATP-sensitive cells are also strongly stimulated by three slowly degradable analogs of ATP, namely, α,β-methylene ATP (AMPCPP), β,γ-methylene ATP (AMPPCP), and β,γ-imido ATP (AMPPNP), (see Fig. 7). The potency sequence is AMPPNP > ATP = AMPPCP > AMPCPP, with the analog AMPPNP being significantly more effective than ATP itself.

Collectively, the above results show that the ATP-sensitive cells in the antennules of the spiny lobster share the following properties with the P_2-type purinoceptors described by Burnstock (*52*). In the antennular receptors, ATP is far more effective than ADP, which in turn is more effective than either AMP or Ado. Secondly, the antennular receptors show a broad sensitivity to nucleotide triphosphates including those with modifications in both the adenine and ribose moieties. This broad sensitivity is consistent with that of P_2-type purinoceptors in brain (*47*) and in smooth muscle of various organs (*62–64*). Thirdly, both the antennular chemoreceptors and P_2-type purinoceptors are stimulated by the slowly degradable analogs of ATP, namely, AMPPNP, AMPPCP, and AMPCPP. Further, the analog AMPPNP is more effective than ATP itself on the

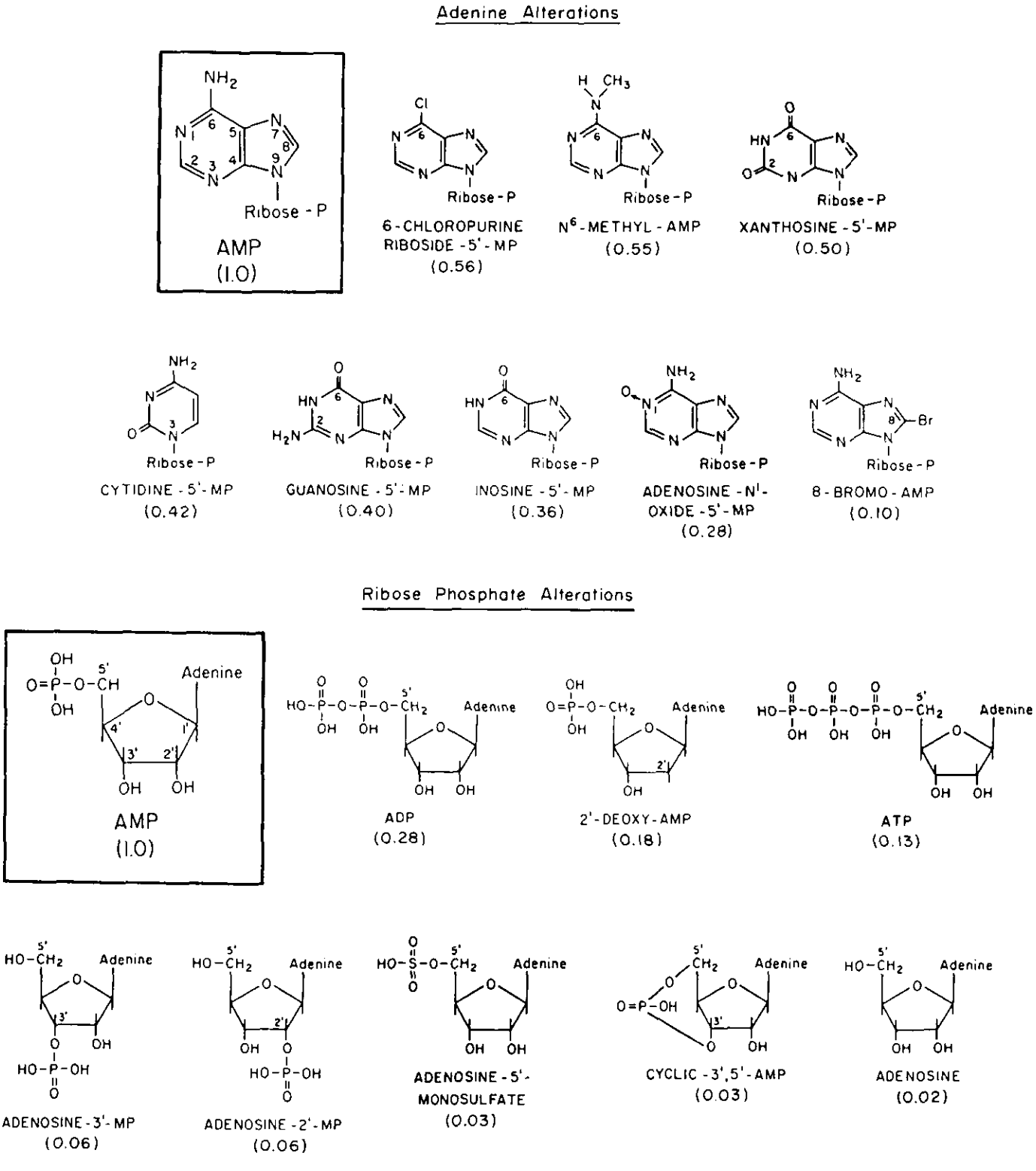

FIGURE 5. Molecular formulae of AMP and analogs tested on AMP-sensitive chemosensory cells of the spiny lobster. Analogs with alterations in the adenine moiety (upper) and the ribose phosphate moiety (lower) were tested. The number beneath each substance represents the intensity of the physiological response to that substance (10 μM) relative to the response to 10 μM AMP. Data from Derby et al. (*37*).

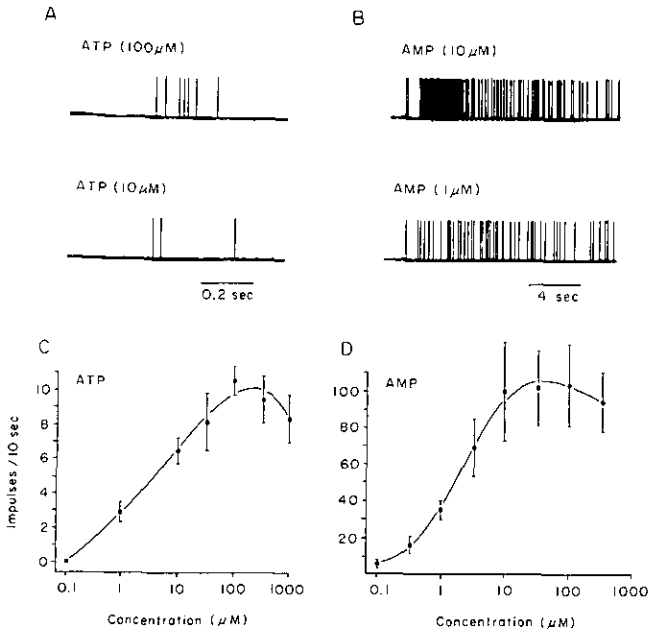

FIGURE 6. Comparisons of response characteristics of ATP-sensitive cells and AMP-sensitive cells in the antennule of the spiny lobster. (*A,B*) Response profiles of the two cell types each tested at two concentrations. Note differences in time scale, plus differences in duration and magnitude of responses. (*C,D*) Dose-response curves for populations of each cell type. Data points are mean values (± SEM). From Carr et al. (*38*).

antennular receptors; this and other analogs are also more effective than ATP on certain P_2 purinoceptors identified in internal tissues (*65,66*).

The role of ATP as an olfactory excitant in the spiny lobster is unknown. However, this substance does have a role as a gustatory stimulant that induces certain blood-sucking insects to gorge on liquid (*67–69*). Moreover, the behavioral responses evoked by ATP in these insects also appear to be mediated by a P_2-like purinoceptor (*38*).

Inactivation and Uptake of Purinergic Substances

Further parallels between purinergic systems found in the lobster olfactory organ and in internal tissues of vertebrates are revealed by comparing the biochemical fate of excitatory nucleotides in both cases. In vertebrate tissues, the inactivation of extracellular nucleotides occurs by a two-step process: first, the nucleotides are dephosphorylated, and then the resultant Ado is internalized by an uptake system (*70*). Dephosphorylation is mediated by ectonucleotidases present on external membrane surfaces of neurons, glial cells, and other cell types (*71–73*). Ado is salvaged by an uptake system believed to involve facilitated diffusion (*74–76*).

In the spiny lobster, sensilla on the antennule also have an ectonucleotidase that inactivates the excitatory nucleotide, AMP, to yield the non-excitatory product, Ado, which is then internalized by an uptake system within the sensilla (Trapido-Rosenthal et al., in preparation). Dephosphorylation is rapid; the rate of uptake of ^3H from ^3H-AMP (labeled in the purine ring) is indistinguishable from the rate of uptake of ^3H from ^3H-Ado (Fig. 8*A*). Evidence that AMP is indeed dephosphorylated prior to uptake is shown by the fact that the rate of uptake of ^3H from ring-labeled AMP is about 10 times greater than that of ^{32}P from ^{32}P-AMP (Fig. 8*B*). Also, the 5′-nucleotidase inhibitor, AMP-C-P, inhibits the uptake of ^3H from ring-labeled AMP but not from labeled Ado (Trapido-Rosenthal et al., in preparation).

Taurinergic Chemoreceptors

Background

Taurine (2-aminoethanesulfonic acid) is found at high concentrations in a wide variety of animal tissues (*77*) and has been the object of increased research effort during the past 15 years (*78–82*). This ubiquitous amino acid has been associated with neural development (*83*), heart function (*84*), retinal function (*85*), osmoregulation (*86,87*), opioid activity (*88,89*), and disease states such as epilepsy (*90*) and Friedreich's ataxia (*91*). Taurine has also been postulated to act as an inhibitory neurotransmitter and/or neuromodulator (*92,93*). Nevertheless, despite taurine's known or suspected involvement in numerous physiological processes, its functional significance remains largely speculative. Unlike other structurally related, bioactive amino acids (e.g., glycine and GABA), the role of taurine is poorly understood because of a paucity of selective agonists and antagonists for pharmacological studies. This lack of selective pharmacological probes is due in large measure to the absence of a model receptor system in vertebrates that is specifically sensitive to taurine (*94*). As described below, taurine-sensitive chemoreceptors of the lobster provide a unique model for exploring taurine reception in detail, and as such may prove invaluable in the identification of pharmacological agents for use in deciphering taurine's role in vertebrate systems.

Lobster Taurinergic Chemoreceptors

Chemoreceptors narrowly tuned to taurine have been identified on the antennules of the spiny lobster, *P. argus* (*33,36*), and on the walking legs and antennules of the American lobster, *H. americanus* (*26,35,95,96*). In *P. argus*, average dose-response relationships for these olfactory cells show that the number of impulses produced, the maximum frequency of discharge, and the response duration all increase in an approximately linear fashion at concentrations between 10^{-9} and 10^{-4} M taurine (*36*). Although threshold differences exist between individual taurine-sensitive cells, single cells are consistent in their response to repeated stimulation. Threshold determinations have revealed responses to taurine at concentrations as low as 10^{-12} M, the lowest dose examined (*33*).

Fuzessery et al. (*36*) investigated the stimulatory ca-

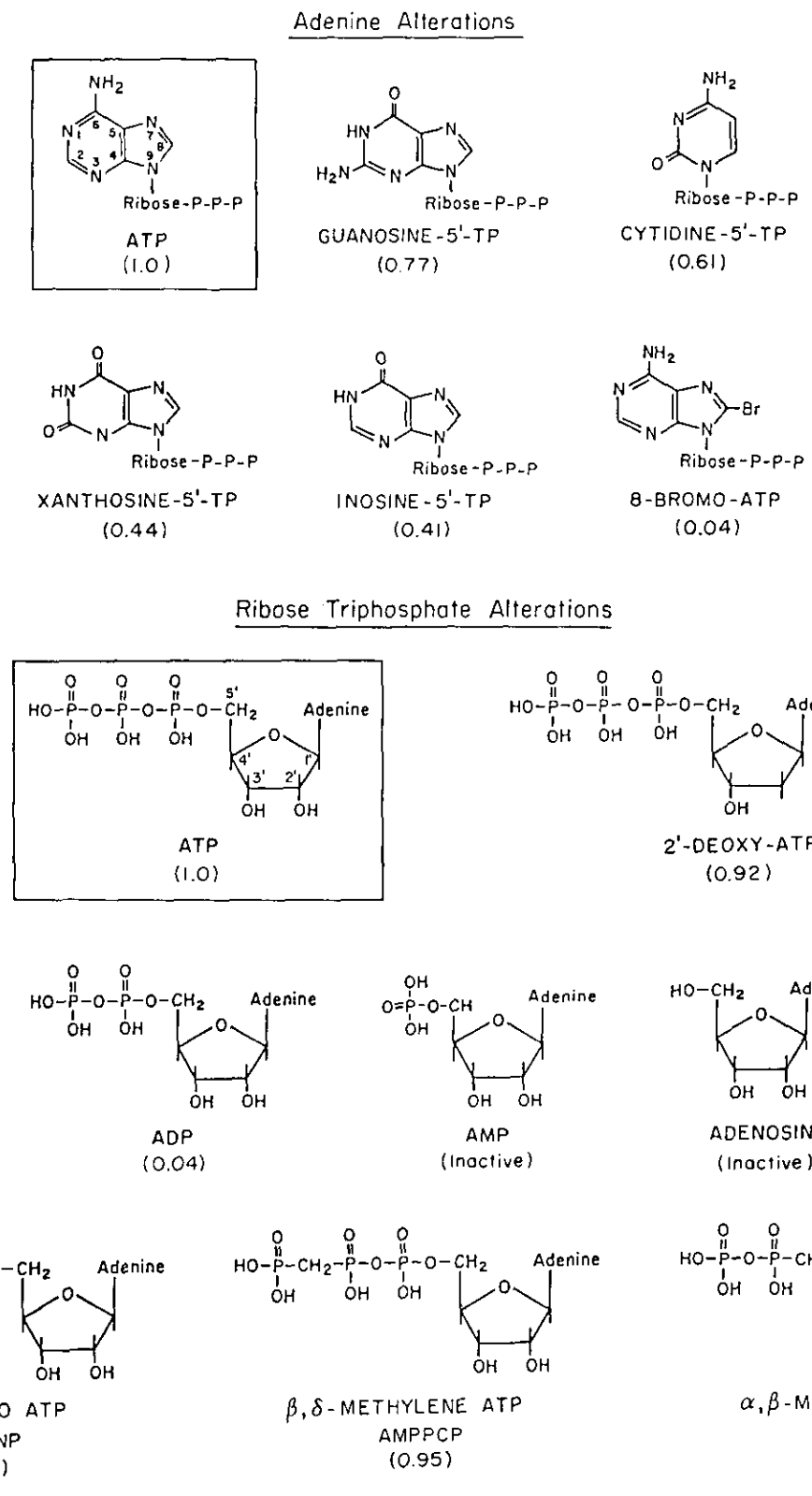

FIGURE 7. Molecular formulae of ATP and analogs tested on P_2-like chemoreceptors of the spiny lobster.

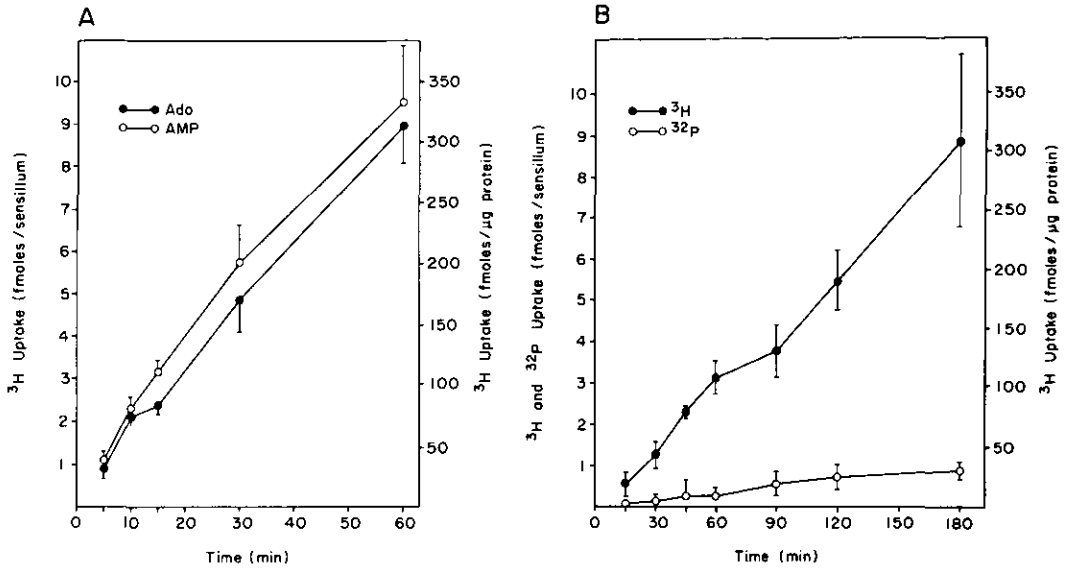

FIGURE 8. Biochemistry of purinergic substances in excised chemosensory sensilla of the spiny lobster. (*A*) Sensilla were incubated with 0.1 µM adenine-labeled ^{3}H-adenosine or ^{3}H-AMP in the presence and absence of an excess (1000 µM) of unlabeled adenosine. The tritiated adenine moiety of AMP and adenosine were internalized at very similar rates. (*B*) Sensilla were incubated in a mixture containing both 0.1 µM ^{32}P-AMP and 0.1 µM adenine-labeled ^{3}H-AMP in the presence and absence of excess unlabeled adenosine. The uptake of ^{3}H but not ^{32}P indicate that most AMP is dephosphorylated prior to uptake. From Trapido-Rosenthal et al., in preparation.

pacity of compounds structurally related to taurine (Fig. 9), each presented at a concentration of 10^{-5} M. These data disclosed the following relationships [Note that numbers in brackets below refer to compound numbers in Fig. 9].

Compounds with one terminal basic group and one terminal acidic group separated by two carbon (C) atoms are the most active [1,2]. An exception occurs when a phosphonic acid group is substituted for the carboxyl group [3]. Compounds with the basic and acidic groups separated by greater than 2C atoms are less stimulatory with potency decreasing as a function of the distance of separation [e.g., 2>15>16>17]. Compounds with a 1C atom separation are not stimulatory [4,14].

Compounds lacking the terminal amino group [5,6,7,9,10] or with an alpha amine group in addition to the terminal amino group are virtually inactive [9,11,12]. Substituting a guanido group for the terminal amine results in loss of activity [20,21].

Neutral side chains markedly decrease the stimulatory capacity [15>18; 2>19].

Compounds with the carboxyl group blocked in a peptide bond are not stimulatory [22,23].

In addition, compounds structurally dissimilar to taurine [twelve α-amino acids, three organic acids (citric, propionic and succinic) and the quaternary amine, glycine betaine] did not stimulate 65 other taurine-sensitive cells on which each compound was tested at 10^{-5} M.

Collectively these findings indicate that the taurine receptor cells in *P. argus* have a very restricted specificity and exhibit interesting similarities in their response spectra to taurine-sensitive systems found in internal tissues of vertebrates. For example, induced arrhythmias in dogs can be suppressed by taurine, but ethanesulfonic acid and other compounds which lack either the basic or acidic group are ineffective (*97*). In human blood platelets, uptake of taurine is competitively inhibited by β-alanine and hypotaurine, but not by the phosphonic acid analog (*98*). Likewise, hypotaurine and β-alanine compete with taurine for uptake in rat brain, while α-amino acids do not (*99,100*). These results from vertebrate internal systems parallel those in the lobster olfactory system; i.e., β-alanine and hypotaurine are the two most active analogs for the taurine-sensitive olfactory cells, whereas α-amino acids, the phosphonic acid analog of taurine, and ethanesulfonic acid are all inactive.

Although not as extensively studied in *H. americanus*, taurine-sensitive chemoreceptors on the antennules (*95,96*) and walking legs (*26,35*) of this species appear to exhibit "narrow tuning" comparable to that found in *P. argus*. For example, in a sample of seven taurine-sensitive cells on the antennules of *H. americanus*, taurine was very effective, but a series of 14 other compounds (L-hydroxyproline, L-glutamate, L-glutamine, ammonium chloride, L-arginine, sucrose, ethanol, L-alanine, L-lysine, betaine, L-aspartate, glycine, L-leucine, and L-proline), each presented at a concentration of 10^{-4} M, showed little or no stimulatory activity (*96*). Interestingly, when taurine was presented in mixture with all 14 of the above substances, the cells' responses were significantly suppressed. Further study of this phenomenon revealed that the suppression was concentration dependent; i.e., with diluted mixtures (each compound $<10^{-6}$ M) cell responses were not sup-

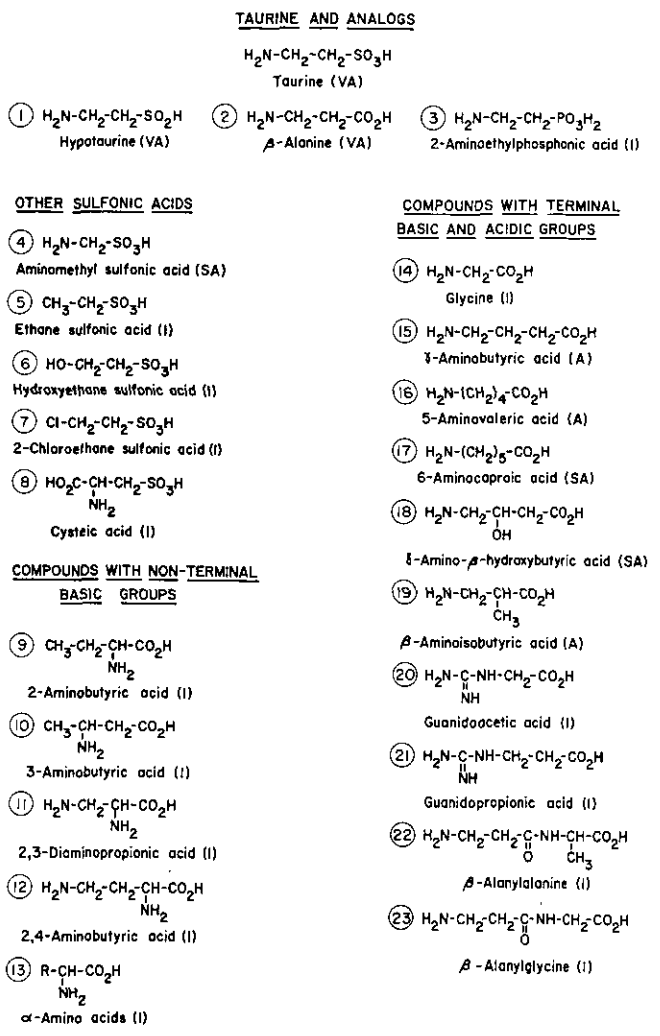

FIGURE 9. Molecular formulae of compounds tested on taurine-sensitive chemoreceptors of spiny lobster. Indices of relative activity are as follows: (VA) very active; (A) active; (SA) slightly active; (I) virtually inactive. Numbers designating compounds are referred to in text. Modified from Fuzessery et al. (*36*).

pressed, and in fact tended to be slightly greater than with taurine presented alone (*29*).

The mechanism of the above mixture suppression is not understood. However, in *P. argus*, studies of suppression on a second type of taurine-sensitive chemosensory cell suggest that competition for common receptor sites may be involved (*101*). The taurine-sensitive cells exhibiting suppression are less sensitive to taurine than the cell population previously described for *P. argus*; these cells have thresholds close to 10^{-5} M, and the dose-response functions have a lesser slope. In these cells, the response to taurine is inhibited when taurine is presented in binary mixture with certain amino acids. Glycine is one of the most effective inhibitors (suppressants), and an analysis of the nature of the inhibition has indicated a parallel shift to the right of the taurine dose-response function. These results are particularly intriguing in the light of taurine's suspected interactions with glycine receptors in some vertebrate preparations (*102–104*).

Glutamatergic Chemoreceptors

Background

For many years the acidic amino acids, glutamate and aspartate, have been candidate excitatory neurotransmitters in both vertebrates and invertebrates (*105–107*). Studies with molluscs and coelenterates have indicated that receptors for these amino acids are present in the CNS and nerve-muscle preparations, respectively. Perhaps the most thoroughly investigated invertebrate group, however, has been the arthropods, where the excitatory potency of glutamate has been shown for neuromuscular junctions in both crustaceans and insects (*105*).

Although the excitatory effects of glutamate in the vertebrate CNS were described over 30 years ago (*108*), the characterization of receptors for this and other structurally related amino acids has progressed slowly. Current neuropharmacological data indicate that multiple receptor types exist for these excitatory compounds (*107,109*). At least three classes of synaptic receptors have been identified, each associated with a different ionophore. One class is activated preferentially by quisqualate and related compounds; L-glutamate and L-cysteate are candidate transmitters for these receptors. A second class is preferentially excited by *N*-methyl-D-aspartate (NMDA); L-aspartate may be a transmitter for this class, but is also an agonist for quisqualate receptors. The third receptor type is distinguished by its activation with kainic acid, the effects of which are unaffected by quisqualate or NMDA receptor antagonists. The endogenous transmitter(s) for the kainate class of receptors is unknown. In addition to these three receptor classes, there is emerging pharmacological evidence for other classes or subtypes, reflecting the unsettled state of understanding in this field (*107,109*).

Lobster Glutamatergic Chemoreceptors

Glutamate sensitive chemoreceptors on both the antennules and walking legs have been described in a number of marine crustaceans (*25,26,95,110–113*). In single unit studies with antennule preparations, a range of cell specificities is apparent. For example, Derby and Ache (*113*) analyzed 70 receptor cells in *P. argus* for responses to eight substances including L-glutamate, taurine, betaine, L-alanine, glycine, AMP, L-proline, and hydroxy-L-proline, each presented at the relative concentrations at which they occur in a natural food stimulus mixture. Eighteen of the cells responded only to glutamate and betaine; 11 of these responded most strongly to glutamate, whereas the remaining seven cells exhibited approximately equal activation by glutamate and betaine. In *H. americanus*, only a single cell was encountered that responded exclusively to L-glutamate when 30 cells

were tested with a battery of 15 substances (*96*). A second glutamate-sensitive cell was equally stimulated by hydroxy-L-proline. Finally, it was reported that a single cell of nine examined in *H. americanus* responded only to L-glutamate when 29 D- and L-amino acids were tested (*95*).

In contrast to the antennular receptors described above, "narrowly tuned" glutamate chemoreceptors appear to comprise a major portion of the chemoreceptor population of the walking legs of the American lobster, *H. americanus* (*26,35*). In experiments examining the structure-activity relationships for activation of these cells, all of the glutamate analogs tested (Fig. 10) had a greatly reduced stimulatory capacity; i.e., responses were less than 8% of that to L-glutamate (*26*). These analogs included the following structural modifications [Note that numbers in brackets refer to compound numbers in Fig. 10]: (1) substituting phosphonic or sulphonic acid groups for the γ-carboxylic acid group [1,2]; (2) α-carbon substitution [3]; (3) isomerization (D-glutamate); (4) deamination [4]; (5) cyclization [16]; (6) changing the carbon chain length [5,6]; (7) altering the α-carboxylic acid group [7,8]; (8) alteration of the γ-carboxylic acid group [9,11,12,13]; (9) altering the carbon chain length as well as the γ-carboxylic acid group [14,15]; and (10) adding a second or third amino acid group [10,18]. Because cysteate [14], aspartate [5], and kainate [17] were all virtually inactive, these results suggest that the walking leg receptors represent a class that is distinctly different from the classes of internal "glutamate" receptors described earlier. Indeed this difference is apparent even within the same species. For example, aspartate potentiates the actions of L-glutamate at the neuromuscular synapse in *H. americanus*, presumably by modulating the affinity of the glutamate receptor (*114*); however, tests exploring similar potentiation with the glutamate chemoreceptors proved negative (*26*). Furthermore, unlike the glutamate chemoreceptor, the neuromuscular preparation in *H. americanus* is stimulated by kainate (*115*).

Glycinergic Chemoreceptors

Glycine is known to act as an inhibitory neurotransmitter in vertebrates, and its role as such is best established in the spinal cord (*116*) where there is good evidence that Renshaw cells are glycinergic (*117*). Although glycine sensitive chemoreceptors have been found in crustaceans, their characterization has lagged behind other receptor types because of difficulties in locating single units. For example, even though glycine was one of the most stimulatory amino acids based on multiunit recordings in the walking legs of *H. americanus* (*25*), a search for single cells was unsuccessful (*26*). Also, within a sample group of 70 chemoreceptor cells on the antennules of *P. argus*, only two exhibited selective sensitivity to glycine (*113*). Recently, a new search protocol in our laboratory, using higher concentrations of glycine, has facilitated the identification of glycine-sensitive cells, and these are currently under study.

Single chemoreceptive cells on crayfish (*Orconectes limosus*) legs have been characterized which are sensitive to a range of amino acids and related substances (*118*). These findings suggest that a single receptor site type, broadly sensitive to amino acids, mediates the responses of these cells. In this system glycine is moderately stimulatory with a K_m of 1.5×10^{-3} mole/L. This broad sensitivity to amino acids in a freshwater crustacean markedly contrasts with the situation for chemoreceptors of marine species, possibly indicating that the different ionic environments to which the chemoreceptors are exposed have given rise to the evolutionary divergence.

Other Chemoreceptors

Antennular chemoreceptors of the California spiny lobster (*P. interruptus*) respond to 10^{-9} to 10^{-13} M concentrations of the steroid molting hormones, α- and β-ecdysone (*119*). Chemoreceptors responding to α-ecdysone do not respond to β-ecdysone, but additional details of receptor specificity are not known. The short latency period of these receptor cells indicates that the electrophysiological response is mediated by a membrane receptor rather than by a cytoplasmic or nuclear event such as characterizes the effects of many other steroids (*119*). Hence the antennular chemoreceptors may prove useful for comparative studies of steroid receptors located on cell surfaces.

Walking legs of crayfish (*Orconectes limosus* and *Austropotamobius torrentium*) have chemoreceptor cells that are sensitive to pyridine analogs but not to amino acids or amines (*40,120*). Tests of 79 pyridine analogs and related compounds on these cells revealed that the most effective substances were pyrazinecarboxamide > 3-acetylpyridine > nicotinamide > pyridine-3-aldoxime. K_m values for the above substances ranged from about 10^{-6} to 4×10^{-4} M (*28,121*). 4-Acetylpyridine was identified as a competitive antagonist of the pyridine-sensitive cells. Although possible relationships between the pyridine-sensitive chemoreceptors of crayfish and internal receptor types have not been explored, the pyridine moiety is indeed represented in many substances known to have regulatory or metabolic effects in internal tissues (e.g., nicotinic acid, nicotinamide, NAD, NADP, and others) (*122*). Also, the pyridine-sensitive chemoreceptors are stimulated by 3- and 4-aminopyridines that are known to have excitatory effects on synaptic and axonal structures (*123,124*).

Possible Evolutionary Implications of External Chemoreceptors Sensitive to Internal Neuroactive Agents

In multicellular aquatic organisms, the principal functions of external chemoreceptors and of internal recep-

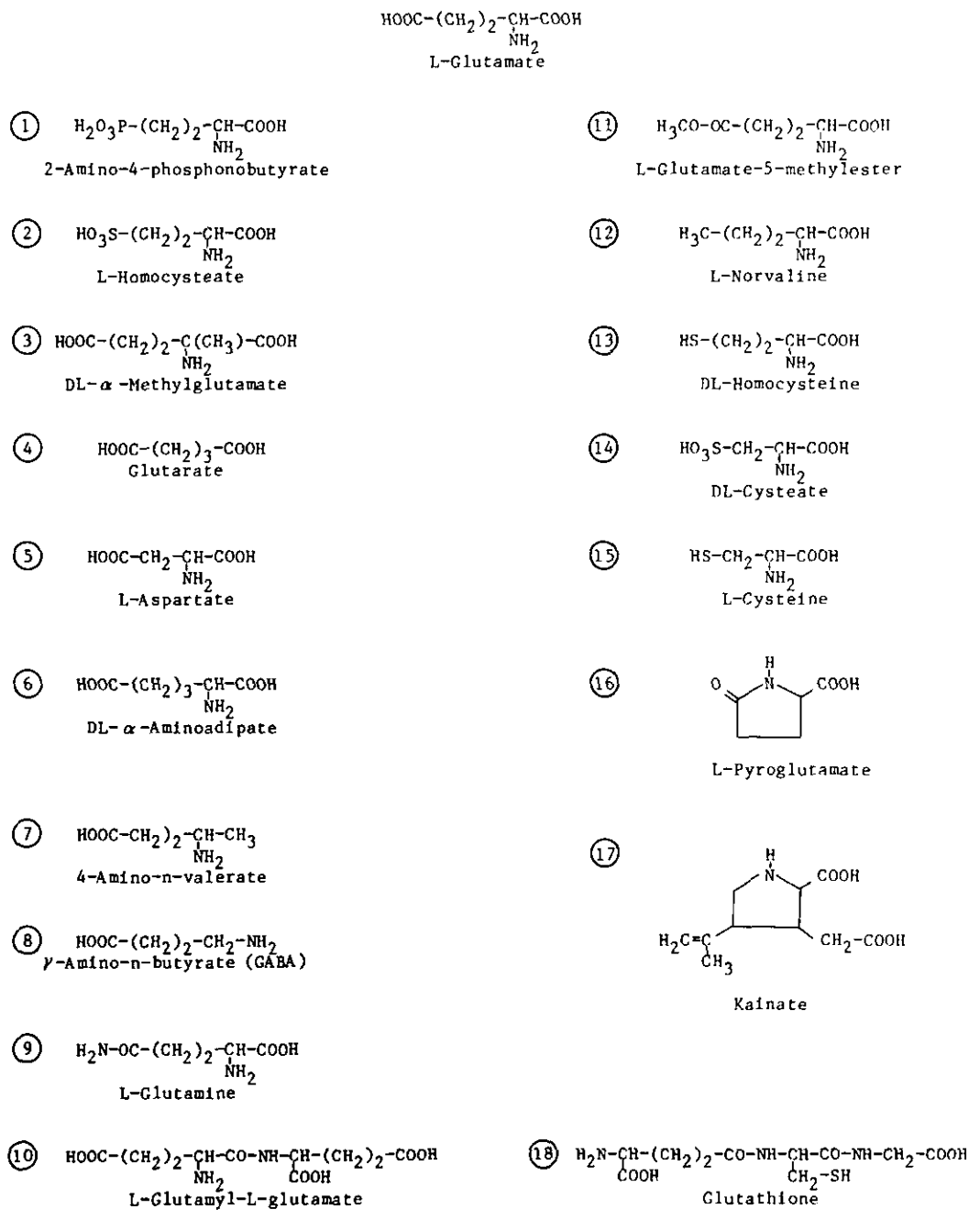

FIGURE 10. Molecular formulae of compounds tested on glutamate-sensitive chemoreceptors of the American lobster. Numbers designating compounds are referred to in text. Formulae from Derby and Atema (*26*).

tors (e.g., synaptic receptors) are very similar in that both monitor particular chemicals in their surrounding aquatic environment. Both types of "chemo"-receptors have binding sites with an affinity for specific molecules, and both are coupled to a transduction mechanism capable of affecting membrane excitability. Indeed, Haldane (*125*) hypothesized that some internal receptor types may have evolved from external chemoreceptors of primitive aquatic animals. Support for this hypothesis is provided by the fact that all of the neuroactive substances listed in Table 1 are known to activate receptors existing both in internal tissues and on external chemosensory surfaces.

Further support for the hypothesis of a common origin for certain external and internal receptor types is indicated by the finding that receptors for the neurotransmitters acetylcholine, catecholamines, and GABA are variously distributed on the surface membranes of oocytes from frogs, mice, and humans (*126,127*). Such receptors are exposed to chemical signals present in an

Table 1. Neuroactive agents for which external chemoreceptors exist.

Substance	Organism with chemoreceptors for substance	Behavior influenced by substance	Reference
AMP	Shrimp, spiny lobster, mosquito larvae	Attraction of shrimp, mosquito larvae	(*37, 53, 58*)
Cyclic AMP	Slime mold	Attraction	(*42, 129*)
ATP	Mosquito, spiny lobster	Liquid ingestion by mosquito	(*38, 68*)
Acetylcholine ACH	Ciliated protozoan	Swimming velocity	(*130*)
γ-Amino-butyric acid (GABA)	Abalone larvae	Settlement and metamorphosis	(*131*)
Dihydroxy-phenylalanine (DOPA)	Oyster larvae	Settlement and metamorphosis	(*132*)
Glutamate	Lobster, colonial algae	Induction of sexual stage in algae	(*26, 133*)
Glycine	Mud snail	Proboscis extension	(*134*)
Taurine	Spiny lobster	Attraction	(*36*)
α, β-Ecdysone	Spiny lobster	Not known	(*119*)
Prostaglandin A2 (PGA_2)	Fish	Inhibition of feeding	(*135*)

external aquatic environment prior to the developmental events that distribute daughter cells into the internal layers of the embryo. In fact, the entire vertebrate nervous system with its many receptor types for transmitters and other neuroactive substances traces its embryological origin to an outer layer of cells, the ectoderm. The ectodermal cells interface with an external aquatic environment until the onset of gastrulation and the further developmental processes that create new internal aquatic interfaces between new neighboring cells, cell layers, and body fluids.

Certainly much can be learned by using the chemoreceptors of appropriate invertebrates as model systems for several receptor types found in internal tissues (*37,128*). Chemoreceptors of large crustaceans such as lobsters are particularly suitable for this purpose because the receptor cells are present on large, easily accessible, external appendages; i.e., the antennules and legs. More importantly, lobsters serve as excellent model systems because they are now known to have discrete chemoreceptors for at least six neuroactive agents, namely, AMP, ATP, glutamate, glycine, taurine and ecdysones (see Table 1). Moreover, techniques now exist to investigate the biochemical (Trapido-Rosenthal et al., in preparation) and biophysical (*30*) processes by which these agents activate chemoreceptor cells.

The fact that chemoreceptors are directly exposed to chemicals appearing in the external aquatic environment implies that the chemosensory system may be particularly vulnerable to foreign chemicals or to other factors that modify the aquatic environment. Environmental variables that are known to influence certain chemosensory responses of aquatic animals include the following: temperature (*136,137*), pH (*138*), seasonality (*139*), feeding state (*139*), detergents (*140*), industrial wastes (*141*), drilling muds (*142*), oil (*143*), and certain heterocyclic compounds (*144*). Aside from recordings of the effects of temperature on pyridine- and amino acid-sensitive units (*136,137*), the effects of these environmental variables upon the discrete types of chemoreceptors described in this review are unknown.

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